

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

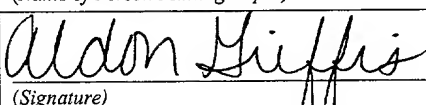
Applicants: Tsien et al. Art Unit: Unassigned  
Application No.: Unassigned Examiner: Unassigned  
Filed: January 25, 2002  
Title: TANDEM FLUORESCENT PROTEIN CONSTRUCTS

Box PATENT APPLICATION  
Commissioner for Patents  
Washington, DC 20231

Sir:

**PRELIMINARY AMENDMENT**

In connection with the filing of the above-identified patent application, which is a Continuation of U.S. Serial No. 09/396,003, filed September 13, 1999, and prior to examination of the subject application, entry of the amendments and consideration of the following remarks respectfully are requested.

<b>CERTIFICATION UNDER 37 CFR §1.10</b> "EXPRESS MAIL" Mailing Label Number: <b>EV 016 236 959 US</b> Date of Deposit: <b>January 25, 2002</b>	
I hereby certify that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" with sufficient postage on the date indicated above and is addressed to: Box PATENT APPLICATION, Commissioner for Patents, Washington, D C. 20231.	
Aldon Griffis (Name of Person Mailing Paper)	
 (Signature)	January 25, 2002 (Date)

## I. AMENDMENTS

### IN THE DRAWINGS

Please enter Substitute Figure 1B and Substitute Figure 2.

### IN THE SPECIFICATION

Please delete the sentence at page 1, lines 3-4, and substitute therefor:

--This application is a continuation of U.S. Serial No. 09/396,003, filed September 13, 1999, which is a continuation of U.S. Serial No. 08/792,553, filed January 31, 1997 (now U.S. Patent No. 5,981,200), which is a continuation-in-part of U.S. Serial No. 08/594,575, filed January 31, 1996.--

### IN THE CLAIMS

Please cancel claims 1 to 56.

Please add new claims 57 to 78 as follows:

- 57. A tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties,

and wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or
- b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- c) Tyr66His and Tyr145Phe, or
- d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
- e) Ser72Ala, Tyr145Phe and Thr203Ile, or
- f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

58. A tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Tyr66His and Tyr145Phe, or
  - b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, and
- the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Cys or
- b) Ser65Thr.

59. The construct of claim 57 or 58, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.
60. The construct of claim 57 or 58, wherein the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.

61. The construct of claim 57 or 58, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV-1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, b-Secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.
62. A recombinant nucleic acid coding for expression of a tandem fluorescent protein construct, the construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties,
- and wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,
- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or
  - b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
  - c) Tyr66His and Tyr145Phe, or
  - d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
  - e) Ser72Ala, Tyr145Phe and Thr203Ile, or
  - f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr, and
- the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,
- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
  - b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

63. A recombinant nucleic acid coding for expression of a tandem fluorescent protein construct, the construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Tyr66His and Tyr145Phe, or
- b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Cys or
- b) Ser65Thr.

64. The nucleic acid of claim 62 or 63, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

65. A host cell transfected with an expression vector comprising an expression control sequence operatively linked to a sequence coding for the expression of a tandem fluorescent protein construct, the construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or
- b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- c) Tyr66His and Tyr145Phe, or
- d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
- e) Ser72Ala, Tyr145Phe and Thr203Ile, or
- f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

66. A host cell transfected with an expression vector comprising an expression control sequence operatively linked to a sequence coding for the expression of a tandem fluorescent protein construct, the construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and

wherein the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Tyr66His and Tyr145Phe, or
- b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Ans212Lys, and

the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Cys or
- b) Ser65Thr.

67. The host cell of claim 65 or 66, further comprising a protease that is not naturally expressed by the host cell.
68. The host cell of claim 65 or 66, wherein the host cell is *E. coli*.
69. The host cell of claim 65 or 66, wherein the host cell is an eukaryotic cell.
70. The host cell of claim 65 or 66, wherein the host cell is a mammalian cell.
71. A method for measuring protease activity in a sample, comprising:
- 1) contacting the sample with the tandem fluorescent protein construct of claim 57 or 58 that comprises a linker moiety comprising a cleavage recognition site specific for the protease;
  - 2) exciting the donor moiety by radiation; and
  - 3) measuring fluorescence resonance energy transfer between the donor and acceptor moieties at a first time and a second time after addition of the tandem fluorescent protein construct whereby a decrease in fluorescence resonance energy transfer upon incubation of the sample with the tandem fluorescent protein construct indicates protease activity.

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72. A method of measuring protease activity in a cell, comprising the steps of:
- 1) providing a cell that expresses the tandem fluorescent protein construct, of claim 57 or 58 that comprises a linker moiety comprising a cleavage recognition site specific for the protease;
  - 2) exciting the donor moiety by radiation; and
  - 3) measuring the degree of fluorescence resonance energy transfer between the donor and acceptor moieties wherein cleavage of the construct by the protease results in less fluorescence resonance energy transfer which reflects protease activity.
73. The method of claim 72, wherein the step of providing a cell comprises; inducing a sudden increase in expression of the tandem fluorescent protein construct, and the step of measuring the degree of fluorescence resonance energy transfer comprises; determining the degree at a first and a second time after induction of tandem fluorescent protein construct expression and determining the difference between the first and second time, whereby less fluorescence resonance energy transfer reflects the presence of the protease.
74. A method for determining whether a compound alters the activity of a protease comprising the steps of:
- contacting a sample containing a known amount of the protease with the compound and with the tandem fluorescent protein construct of claim 57 or 58;
  - exciting the donor moiety by radiation; and
  - determining the degree of fluorescence resonance energy transfer between the donor and acceptor moieties in the sample containing the compound, and comparing the degree of fluorescence resonance energy transfer between the donor and acceptor moieties in a sample not containing the compound, whereby a difference in the degree of fluorescence resonance energy transfer indicates that the compound alters the activity of the protease.



75. A method for determining whether a compound alters the activity of a protease in a cell, comprising the steps of:
- 1) providing first and second cells that express the tandem fluorescent protein construct of claims 57 or 58, wherein the linker moiety comprises a cleavage recognition amino acid sequence specific for the protease;
  - 2) contacting the first cell with an amount of the compound;
  - 3) contacting the second cell with a different amount of the compound, or a buffer control;
  - 4) exciting the donor moiety in the first and second cell by radiation;
  - 5) determining the degree of fluorescence resonance energy transfer in the first and second cells; and
  - 6) comparing the degree of fluorescence resonance energy transfer in the first and second cells, whereby a difference in the degree of fluorescence resonance energy transfer indicates that the compound alters the activity of the protease.
76. A tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and wherein the donor or acceptor moieties comprises either,
- an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223 and attenuates the intermolecular interactions between the donor or acceptor moieties,
  - or the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,
- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or

- b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
  - c) Tyr66His and Tyr145Phe, or
  - d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
  - e) Ser72Ala, Tyr145Phe and Thr203Ile, or
  - f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr,
- or the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,
- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
  - b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

77. A recombinant nucleic acid coding for expression of a tandem fluorescent protein construct, the construct comprising construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and wherein the donor or acceptor moieties comprises either,
- an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223 and attenuates the intermolecular interactions between the donor or acceptor moieties,
  - or the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,
- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or
  - b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
  - c) Tyr66His and Tyr145Phe, or
  - d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
  - e) Ser72Ala, Tyr145Phe and Thr203Ile, or
  - f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr,

or the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.

78. A host cell transfected with an expression vector comprising an expression control sequence operatively linked to a sequence coding for the expression of a tandem fluorescent protein construct, the construct comprising construct comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer ("FRET") when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties, and wherein the donor or acceptor moieties comprises either,

an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223 and attenuates the intermolecular interactions between the donor or acceptor moieties,

or the donor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys, or
- b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- c) Tyr66His and Tyr145Phe, or
- d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys, or
- e) Ser72Ala, Tyr145Phe and Thr203Ile, or
- f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr,

or the acceptor moiety comprises an *Aequorea* fluorescent protein (SEQ. ID. No. 2) comprising the amino acid substitutions,

- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr, or
- b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr.--

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## II. REMARKS

Formal Drawings are submitted herewith to replace those originally filed with the parent application. With respect to the Formal Drawings, Figures 1B and 2 have been amended to correct typographical errors. Figure 1B was amended such that the nucleotide position indicated as "717" at the end of the sequence was changed to "716" (see original Figure 1), which is the correct number of nucleotides shown (see, also, SEQ ID NO:1). Figure 2 was amended to correct a misspelling of the term "acid." Marked versions of original Figure 1 and of Formal Drawing Figures 1A and 2 showing the amendments are attached as Exhibit A.

The specification has been amended to update the continuing information. As such, the amendment merely addresses a formality and does not add new matter.

Applicants have cancelled claims 1 to 57 and added new claims 58 to 78. The new claims do not introduce new matter and fully supported by the specification as originally filed. Specific support for the new claims is summarized in the Table below.

Claim Number	Support in Specification
57	Claims 1, 2, 4, 5, Table 1 page 16
58	Claims 1, 2, 3, 4, 5, 9, Table 1 page 16
59	Claim 7
60	Claim 6
61	Claim 10
62	Claims 16, 17,18, Table 1, page 16, pages 31 to 33
63	Claims 16, 17,18, Table 1, page 16, pages 31 to 33
64	Claim 7
65	Claim 22
66	Claim 22

Claim Number	Support in Specification
67	Claim 23
68	Claim 24
69	Claim 25
70	Claim 26
71	Claims 27 to 35, pages 35 to 40
72	Claims 36 to 39, pages 35 to 40
73	Claim 40
74	Claim 42, 44
75	Claim 45
76	Page 19, lines 20 to 33, Page 20 lines 5 to 7, Page 16 Table 1, claims 1, 2, 4, 5,
77	Page 19, lines 20 to 33, Page 20 lines 5 to 7, Page 16 Table 1, claims 16, 17,18
78	Page 19, lines 20 to 33, Page 20 lines 5 to 7, Page 16 Table 1, claims 22 to 24

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In view of the foregoing, Applicants respectfully submit that the claims are ready for examination and are in condition for allowance. Please apply any charges not covered, or any credits, to Deposit Account 50-1355. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Respectfully submitted,

Date: January 25, 2002



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Parameter	Value	Unit
Initial concentration	1.0	mol/L
Temperature	25	°C
Time	0, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096, 8192, 16384, 32768, 65536, 131072, 262144, 524288, 1048576, 2097152, 4194304, 8388608, 16777216, 33554432, 67108864, 134217728, 268435456, 536870912, 1073741824, 2147483648, 4294967296, 8589934592, 17179869184, 34359738368, 68719476736, 137438953472, 274877906944, 549755813888, 1099511627776, 2199023255552, 4398046511104, 8796093022208, 17592186044416, 35184372088832, 70368744177664, 140737488355328, 281474976710656, 562949953421312, 1125899906842624, 2251799813685248, 4503599627370496, 9007199254740992, 18014398509481984, 36028797018963968, 72057594037927936, 144115188075855872, 288230376151711744, 576460752303423488, 1152921504606846976, 2305843009213693952, 4611686018427387904, 9223372036854775808, 18446744073709551616, 36893488147419103232, 73786976294838206464, 147573952589676412928, 295147905179352825856, 590295810358705651712, 1180591620717411303424, 2361183241434822606848, 4722366482869645213696, 9444732965739290427392, 18889465931478580854784, 37778931862957161709568, 75557863725914323419136, 151115727451828646838272, 302231454903657293676544, 604462909807314587353088, 1208925819614629174706176, 2417851639229258349412352, 4835703278458516698824704, 9671406556917033397649408, 19342813113834066795298816, 38685626227668133590597632, 77371252455336267181195264, 154742504910672534362390528, 309485009821345068724781056, 618970019642690137449562112, 1237940039285380274899124224, 2475880078570760549798248448, 4951760157141521099596496896, 9903520314283042199192993792, 19807040628566084398385987584, 39614081257132168796771975168, 79228162514264337593543950336, 158456325028528675187087900672, 316912650057057350374175801344, 633825300114114700748351602688, 1267650600228229401496703205376, 2535301200456458802993406410752, 5070602400912917605986812821504, 10141204801825835211973625643008, 20282409603651670423947251286016, 40564819207303340847894502572032, 81129638414606681695789005144064, 162259276829213363391578010288128, 324518553658426726783156020576256, 649037107316853453566312041152512, 1298074214633706907132624082305024, 2596148429267413814265248164610048, 5192296858534827628530496329220096, 10384593717069655257060992658440192, 20769187434139310514121985316880384, 41538374868278621028243970633760768, 83076749736557242056487941267521536, 166153499473114484112975882535043072, 332306998946228968225951765070086144, 664613997892457936451903530140172288, 1329227995784915872903807060280344576, 2658455991569831745807614120560689152, 5316911983139663491615228241121378304, 10633823966279326983230456482242756608, 21267647932558653966460912964485513216, 42535295865117307932921825928971026432, 85070591730234615865843651857942052864, 170141183460469231731687303715884105728, 340282366920938463463374607431768211456, 680564733841876926926749214863536422912, 1361129467683753853853498429727072845824, 2722258935367507707706996859454145691648, 5444517870735015415413993718908291383296, 10889035741470030830827987437816582766592, 21778071482940061661655974875633165533184, 43556142965880123323311949751266331066368, 87112285931760246646623899502532662132736, 174224571863520493293247799005065324265472, 348449143727040986586495598010130648530944, 696898287454081973172991196020261297061888, 1393796574908163946345982392040522594123776, 2787593149816327892691964784081045188247552, 5575186299632655785383929568162090376495104, 11150372599265311570767859136324180752990208, 22300745198530623141535718272648361505980416, 44601490397061246283071436545296723011960832, 89202980794122492566142873090593446023921664, 178405961588244985132285746181186892047843328, 356811923176489970264571492362373784095686656, 713623846352979940529142984724747568191373312, 1427247692705959881058285969449495136382746624, 2854495385411919762116571938898990272765493248, 5708990770823839524233143877797980545530986496, 11417981541647679048466287755595961091061972992, 228359630832953580969325755111919221821239	

~~717~~  
716

FIGURE 1



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GAT Asp 130	TTT Phe 130	AAA Lys	GAA Glu	GAT Asp	GGA Gly	AAC Asn 135	ATT Ile	CTT Leu	GGA Gly	CAC His	AAA Lys 140	TTG Leu	GAA Glu	TAC Tyr	AAC Asn	432
TAT Tyr 145	AAC Asn	TCA Ser	CAC His	AAT Asn	GTA Val 150	TAC Tyr	ATC Ile	ATG Met	GCA Ala	GAC Asp 155	AAA Lys	CAA Gln	AAG Lys	AAT Asn	GGA Gly 160	480
ATC Ile	AAA Lys	GTT Val	AAC Asn	TTC Phe 165	AAA Lys	ATT Ile	AGA Arg	CAC His	AAC Asn 170	ATT Ile	GAA Glu	GAT Asp	GGA Gly	AGC Ser 175	GTT Val	528
CAA Gln	CTA Leu	GCA Ala	GAC Asp 180	CAT His	TAT Tyr	CAA Gln	CAA Gln	AAT Asn 185	ACT Thr	CCA Pro	ATT Ile	GGC Gly	GAT Asp 190	GGC Gly	CCT Pro	576
GTC Val	CTT Leu	TTA Leu 195	CCA Pro	GAC Asp	AAC Asn	CAT His	TAC Tyr 200	CTG Leu	TCC Ser	ACA Thr	CAA Gln	TCT Ser 205	GCC Ala	CTT Leu	TCG Ser	526
AAA Lys	GAT Asp 210	CCC Pro	AAC Asn	GAA Glu	AAG Lys	AGA Arg 215	GAC Asp	CAC His	ATG Met	GTC Val	CTT Leu 220	CTT Leu	GAG Glu	TTT Phe	GTA Val	672
ACA Thr 225	GCT Ala	GCT Ala	GGG Gly	ATT Ile	ACA Thr 230	CAT His	GGC Gly	ATG Met	GAT Asp	GAA Glu 235	CTA Leu	TAC Tyr	AAA Lys	TA		<del>717</del> 716

FIG. 1B

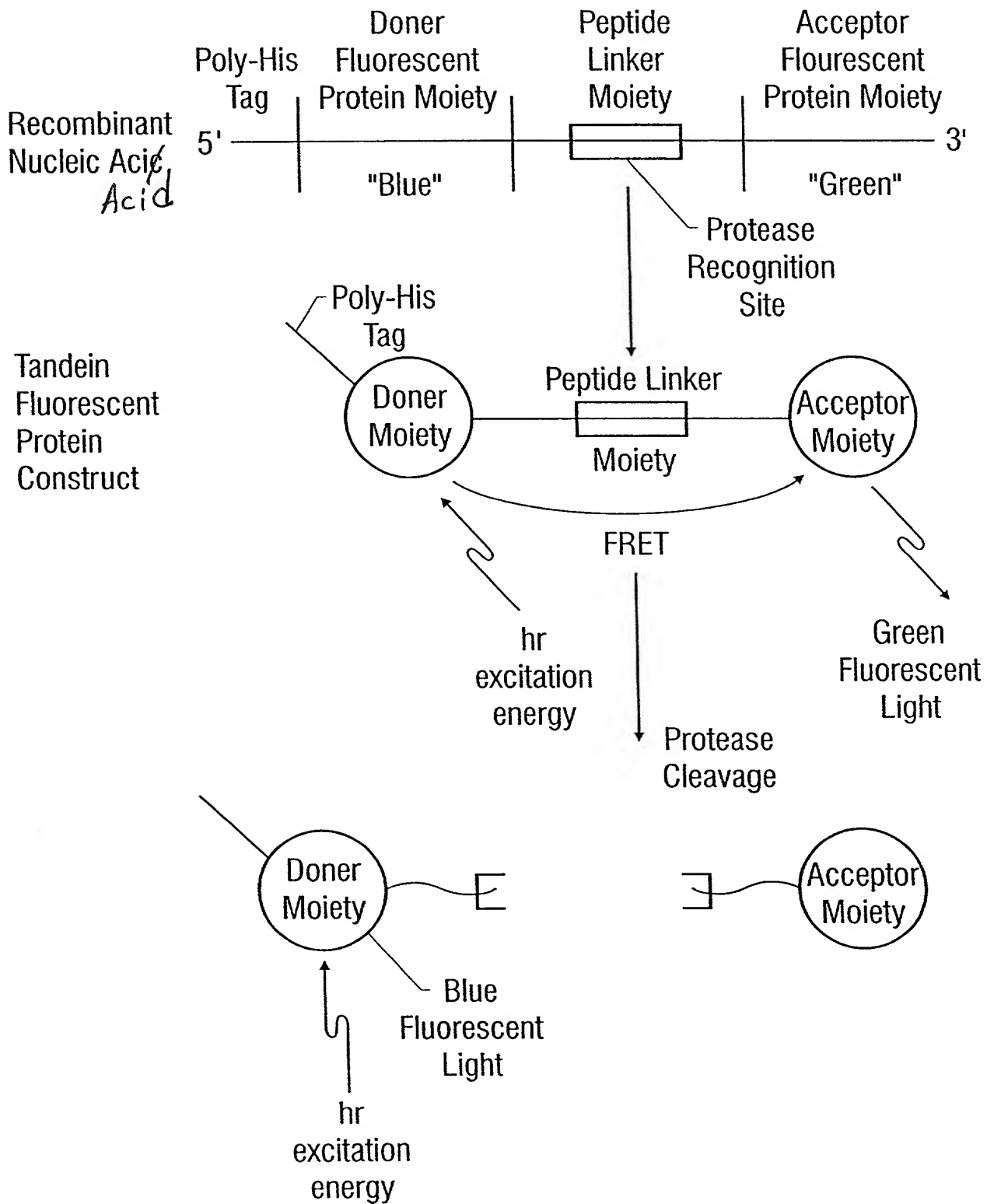


FIG. 2